Applied BioCode

Performance Characterization of a Respiratory Pathogen Panel with an Automated High-throughput System

Sheema Mir, Colleen Knoth, Laya Hodaei, Kassturi Jeevaprakash, Kristen Haag, Michael Aye*

Applied BioCode, Inc., Santa Fe Springs, CA 90670, USA

Abstract

Introduction

Viral and bacterial respiratory tract infections are the most common diseases affecting human worldwide. Therefore, rapid diagnosis of respiratory pathogens may aid to initiate appropriate therapy, reduce unnecessary treatment, decrease hospital stay and reduce overall costs. Applied BioCode's FDA cleared BioCode® MDx-3000, an automated PCR, post-PCR sample handling and detection system in a 96-well format, was used for the verification studies of the BioCode® Respiratory Pathogen Panel (RPP) for simultaneous detection of Influenza A (subtypes H1, H1N1 2009 pdm, H3), Influenza B, Parainfluenza virus (type 1, 2, 3, 4), Metapneumovirus, RSV, Rhinovirus/Enterovirus, Coronavirus (OC43, NL63, 229E, HKU1), Adenovirus, *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis*.

Williams, B.G., Gouws, E., Boschi-Pinto, C., Bryce, J., Dye, C., 2002. Estimates of worldwide distribution of child deaths from acute respiratory infections. Lancet Infect. Dis. 2, 25-32.

Materials & Methods

Following automated extraction (NUCLISENS® easyMAG® or MagNA Pure 96 System) of nucleic acids from nasopharyngeal swab (NPS) specimens, DNA and RNA targets are amplified in a one-step reverse transcription-polymerase chain reaction (RT-PCR). PCR products are captured by target-specific probes coupled to barcoded magnetic beads (BMBs) and fluorescent signal is generated by incubation with a conjugate (Figure 1). Qualitative results are determined by a median fluorescent index (MFI) value relative to assay cutoff.

Conclusions

The BioCode® RPP on the BioCode® MDx-3000 specifically and reproducibly detects viruses and bacteria known to cause upper respiratory tract infections. Once cleared by FDA, our automated system together with our BioCode® Respiratory Pathogen Panel will allow users to perform highly multiplex molecular detection in a high throughput, automated format with simple workflow and minimal hands on time (Figure 2).

- ❖ Clinical performance tested with a set of 503 archived frozen NPS samples compared to an FDA Cleared NAAT and PCR/Sequencing, gave overall 96.9% positive and 99.6% negative agreement (Table 1).
- ❖ Limit of Detections were determined for 24 organisms (Table 2). All were within 5 fold for two extraction systems except ADV C (15 fold).
- ❖ Reproducibility testing on a representative panel for three days with two operators yielded ≥95% positive agreement at 1.5x LoD and ≥99% positive agreement at 3.0x LoD on all targets tested except for HRV with 86.1% & 94.4% (Table 3).
- ❖ No interference was observed with interfering substance panel tested (Table 4).
- ❖ 119 organisms/strains were assessed for inclusivity (Table 5).

Barcoded Magnetic Bead (BMB) Technology

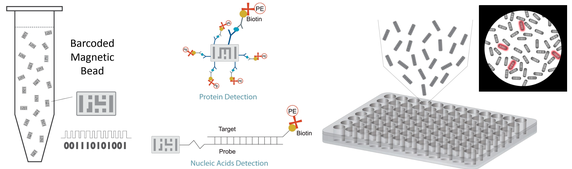

Figure 1. Barcoded Magnetic Beads (BMBs) are coupled to proteins or nucleic acid probes and used for target capture in microtiter plates. For BioCode® RPP, biotinylated PCR products are captured by target-specific nucleic acid probes coupled to BMBs and then labeled by SA-PE for detection.

BioCode® MDx-3000

Figure 2. Workflow for BioCode® RPP. 192 samples in an 8 hour shift with minimal hands on time for MDx-3000. Three different BioCode panels can be performed simultaneously in one plate.

Method Comparison Study

Table 1. Comparison of BioCode® RPP results vs FDA cleared NAAT or PCR/Sequencing (N=501 samples)

Target	Positive Percent Agreement		Negative Percent Agreement	
	n/N	%	n/N	%
Influenza A	84/86	97.7%	410/415	98.8%
Influenza A H1	0/0	N/A	501/501	100.0%
Influenza A H1N1 pdm09	34/34	100.0%	466/467	99.8%
Influenza A H3	50/50	100.0%	446/448	99.6%
Influenza B	39/39	100.0%	459/462	99.4%
Respiratory Syncytial Virus	49/50	98.0%	450/451	99.8%
Human Metapneumovirus	37/37	100.0%	463/464	99.8%
Parainfluenza Virus 1	22/22	100.0%	479/479	100.0%
Parainfluenza Virus 2	15/16	93.8%	485/485	100.0%
Parainfluenza Virus 3	32/32	100.0%	468/469	99.8%
Parainfluenza Virus 4	11/11	100.0%	490/490	100.0%
Adenovirus	52/52	100.0%	436/449	97.1%
Coronavirus 229E	25/32	78.1%	469/469	100.0%
Coronavirus HKU1	10/10	100.0%	491/491	100.0%
Coronavirus NL63	18/18	100.0%	482/483	99.8%
Coronavirus OC43	23/25	92.0%	473/476	99.4%
Human Rhinovirus/Enterovirus	53/59	89.8%	438/442	99.1%
Bordetella pertussis	10/10	100.0%	487/491	99.2%
Chlamydophila pneumoniae	23/23	100.0%	478/478	100.0%
Mycoplasma pneumoniae	10/10	100.0%	491/491	100.0%
Overall agreement	*597/616*	*96.9%*	*9362/9401*	*99.6%*

- All extractions for this study was done on easyMAG®.
- Abstract stated 503 samples, but 2 samples were removed due to operator error.

Limit of Detection (LoD)

Table 2. Limit of Detection (LoD) for the BioCode® RPP performed on the BioCode® MDx-3000 system

Target	Species/Strain/Isolate	easyMAG®	MP96
Influenza A H1	A/New Caledonia/20/99	45 $TCID_{50}$/mL	45 $TCID_{50}$/mL
	A/NWS/33	45 $TCID_{50}$/mL	9.0 $TCID_{50}$/mL
Influenza A H1N1 2009 pdm	A(H1N1)/CA/07/09	1.8 $TCID_{50}$/mL	0.36 $TCID_{50}$/mL
Influenza A H3	A/Wisconsin/67/05	4.0 $TCID_{50}$/mL	4.0 $TCID_{50}$/mL
	A/Alice	9.0 $TCID_{50}$/mL	9.0 $TCID_{50}$/mL
Influenza B	Flu B/Florida/4/2006 (Yamagata)	0.04 $TCID_{50}$/mL	0.04 $TCID_{50}$/mL
	B/Hong Kong/S/1972 (Victoria)	1.8 $TCID_{50}$/mL	9.0 $TCID_{50}$/mL
Respiratory Syncytial Virus	Type A	1.0 $TCID_5$/mL	1.0 $TCID_{50}$/mL
Human Metapneumovirus	16; Type A1 IA10-2003	45 $TCID_5$/mL	45 $TCID_{50}$/mL
Parainfluenza Virus 1	[C-35/Washington DC/1957]	9.0 $TCID_{50}$/mL	45 $TCID_{50}$/mL
Parainfluenza Virus 2	[Greer/Ohio/1955]	9.0 $TCID_{50}$/mL	1.8 $TCID_{50}$/mL
Parainfluenza Virus 3	N/A	15 $TCID_{50}$/mL	15 $TCID_{50}$/mL
Parainfluenza Virus 4	Type 4a	9.0 $TCID_{50}$/mL	9.0 $TCID_{50}$/mL
Adenovirus	Species B Serotype 7A	0.36 $TCID_{50}$/mL	1.8 $TCID_{50}$/mL
Adenovirus	Species C Serotype 2	2.0 $TCID_{50}$/mL	30 $TCID_{50}$/mL
Adenovirus	Species E Serotype 4	0.4 $TCID_{50}$/mL	0.4 $TCID_{50}$/mL
Coronavirus 229E	N/A	1.8 $TCID_{50}$/mL	1.8 $TCID_{50}$/mL
Coronavirus HKU1	N/A	100 copies/RXN	100 copies/RXN
Coronavirus NL63	N/A	0.4 $TCID_{50}$/mL	0.4 $TCID_{50}$/mL
Coronavirus OC43	N/A	0.04 $TCID_{50}$/mL	0.04 $TCID_{50}$/mL
Human Rhinovirus	Type A1	0.36 $TCID_{50}$/mL	0.36 $TCID_{50}$/mL
Bordetella pertussis	A639	45 CFU/mL	45 CFU/mL
Chlamydophila pneumoniae	AR39	50 CFU/mL	50 CFU/mL
Mycoplasma pneumoniae	M129	5.0 CFU/mL	15 CFU/mL

Interference Panel Tested

Table 3. List of interfering substances and microorganisms tested with BioCode® RPP using two extraction systems.

Interfering Substances/Organisms	Concentration/Quantity
Streptococcus pneumoniae	1 X 10^6 CFU/ml
Haemophilus influenzae	1 X 10^6 CFU/ml
Neisseria meningitidis	1 X 10^6 CFU/ml
Staphylococcus aureus	1 X 10^6 CFU/ml
Nasal spray	1% V/V
Nasal decongestant spray	1% V/V
Nasal Allergy spray(Fluticasone)	1.5% V/V
Petroleum Jelly	1% W/V
Analgesic Ointment	1% W/V
Bleach(10%)	5% V/V
Disinfecting wipes	50% V/V
Ethanol(70%)	7% V/V
Copan FloQ (Flocked nylon/plastic shaft)	1 swab
Genomic DNA	15, 10, 5, 2.5 ng/ul
Mucin	0.8, 0.6, 0.3 %W/V

Reproducibility Study

Table 4. Reproducibility for 6 pools (each contains two medium and two low positives) and a negative sample tested over three days by two operators on NUCLISENS® easyMAG®.

Organism	Test Level	Agreement with expected results
Human Rhinovirus Type A1	Medium Positive	94.4% (34/36)
	Low Positive	86.1% (31/36)
	Negative	100% (178/178)
Parainfluenza Virus 2 [Greer/Ohio/1955]	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Human Metapneumovirus (HMPV-16)	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Bordetella pertussis	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Influenza B Florida/4/2006 (Yamagata)	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Coronavirus NL63	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Chlamydophila pneumoniae (TW-183)	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Parainfluenza Virus 3	Medium Positive	100% (36/36)
	Low Positive	100% (36/36)
	Negative	100% (178/178)
Influenza A H3 (A/Wisconsin/67/05)	Medium Positive	100% (36/36)
	Low Positive	100% (35/35)
	Negative	99.4 (178/179)
Mycoplasma pneumoniae (M129)	Medium Positive	100% (36/36)
	Low Positive	100% (35/35)
	Negative	100% (179/179)
Respiratory Syncytial Virus (Type A)	Medium Positive	100% (35/35)
	Low Positive	100% (36/36)
	Negative	100% (179/179)
Adenovirus 2 (Type C)	Medium Positive	100% (35/35)
	Low Positive	100% (36/36)
	Negative	100% (179/179)

Inclusivity

Table 5. List of Strains/Organisms tested with BioCode® RPP as part of inclusivity study.

Target	Number of Strains tested
Adenovirus	17
Influenza A H1	12
Influenza A H3	13
Influenza A H1N1 2009 pdm	8
Influenza B	14
Parainfluenza	9
Coronavirus	1
Human Metapneumovirus	7
Respiratory Syncytial Virus	8
Human Rhinovirus	24
Chlamydophila pneumonia	2
Mycoplasma pneumoniae	4

*Corresponding author: maye@apbiocode.com

The BioCode® Respiratory Pathogen Panel is currently in Clinical Trial.